

# Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Navicula pelliculosa*

PMRA Submission Number {.....}

EPA MRID Number 49760109

<b>Data Requirement:</b>	PMRA DATA CODE {.....}
	EPA DP Barcode 434669
	OECD Data Point {.....}
	EPA MRID 49760109
	EPA Guideline 850.4500

**Test material:** Dithiopyr technical **Purity:** 94.9%

Common name: Dithiopyr

Chemical name: IUPAC: Not reported

CAS name: 3,4-Pyridinecarbothioic acid, (2-difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-S-S-dimethyl ester

CAS number: Not reported

Synonyms: Not reported

**Primary Reviewer:** Dana Worcester  
Environmental Scientist, CDM/CSS-Dynamac JV

Signature:   
Date: 2/27/2017

**Secondary Reviewer:** John Marton, Ph.D.  
Environmental Scientist, CDM/CSS-Dynamac JV

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Jennifer Connolly  
GIS Biologist, EPA/OPP/EFED/EISB

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This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel

**Reference/Submission No.:** {.....}

<b>Company Code</b>	{.....} [For PMRA]
<b>Active Code</b>	{.....} [For PMRA]
<b>Use Site Category:</b>	{.....} [For PMRA]
<b>EPA PC Code</b>	128994

**Date Evaluation Completed:** 6/6/18

**CITATION:** Arnie, J.R., L.A. Lockard, J.R. Porch and K.H. Martin. Dithiopyr TGAI: A 96-Hour Toxicity Test with Freshwater Diatom (*Navicula pelliculosa*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland, USA. Laboratory Project Number: 379P-115. Study sponsored by Dow AgroSciences, LLC, Indianapolis, Indiana, USA. Dow AgroSciences Study Number: 150427. Study initiated May 11, 2015 and completed October 16, 2015.

**DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic nonvascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the

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totality of factors related to the test methodology and results in determining the acceptability of the study.

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## **EXECUTIVE SUMMARY:**

In a 96-hour toxicity study, cultures of freshwater diatom, *Navicula pelliculosa* were exposed to dithiopyr at nominal concentrations of 0 (negative control), 4.3, 9.4, 21, 45, and 100 µg/L under static conditions. Geometric mean measured concentrations were <0.313 (<LOQ, negative control), 2.7, 5.8, 14, 29, and 65 µg ai/L. No solvent was used in this study. The test substance declined substantially during the study, with 96-hour recoveries ranging from 43 to 66% of 0-hour concentrations. Therefore, the reviewer based toxicity values on initial measured concentrations which were <0.313 (<LOQ, negative control), 3.96, 8.26, 20.8, 40.3, and 79.5 µg ai/L.

After 96 hours, NOAEC values for yield and growth rate were 40.3 µg ai/L and for area under the growth curve (AUC) was 20.8 µg ai/L. IC<sub>50</sub> values for growth rate and AUC were 68 and 45.5 µg ai/L, respectively. An IC<sub>50</sub> value for yield could not be calculated. The % growth inhibition of cell density in the treated algal culture as compared to the negative control ranged from -23.5 to 98.8%.

No morphological abnormalities were noted. There were increases in pH during the test. There were no compound-related phytotoxic effects.

This study is scientifically sound and is classified as acceptable.

## **Results Synopsis**

Test Organism: Freshwater diatom, *Navicula pelliculosa* (strain not reported)

Test Type (Flow-through, Static, Static Renewal): Static

Note: N/A = not available

### *Yield*

IC<sub>05</sub>: 35.8 µg ai/L 95% C.I.: 0 to 40.3 µg ai/L

IC<sub>50</sub>: Not calculable 95% C.I.: N/A

NOAEC: 40.3 µg ai/L

Probit Slope: N/A

### *Growth rate*

IC<sub>05</sub>: 44 µg ai/L 95% C.I.: 0 to 49.4 µg ai/L

IC<sub>50</sub>: 68 µg ai/L 95% C.I.: 64.6 to 71.7 µg ai/L

NOAEC: 40.3 µg ai/L

Probit Slope: N/A

### *Area under the curve*

IC<sub>05</sub>: 31.2 µg ai/L 95% C.I.: 0 to 39.5 µg ai/L

IC<sub>50</sub>: 45.5 µg ai/L 95% C.I.: 32.1 to 64.5 µg ai/L

NOAEC: 20.8 µg ai/L

Probit Slope: N/A

Endpoint(s) Effected: Yield, growth rate, and area under the curve.

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## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** The study was performed according to the procedures of OCSPP 850.4500 (2012) and OECD Guideline 201 (2006). The following deviations from the U.S. EPA OCSPP 850.4500 (2012) guideline are noted:

1. The strain of the test organism was not reported.
2. Test concentrations declined substantially during the study, with 96-hour measured concentrations ranging from 43 to 66% of their initial measured counterparts.
3. The pH, TOC, particulate matter, and metals, and chlorine concentrations of the dilution water were not reported.
4. The physico-chemical properties of the test material were not reported.
5. The initial cell density ( $0.5 \times 10^4$  cells/mL) was lower than recommended ( $1.0 \times 10^4$  cells/mL).

These deviations do not impact the acceptability of the study.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The study was performed in accordance with the GLP standards of U.S. EPA (40 CFR Parts 160 and 792), OECD GLP, and Japan MAFF with the following exception: periodic analyses of well water for potential contaminants were not performed according to Good Laboratory Standards, but were performed using a certified laboratory and standard US EPA analytical methods.

## A. MATERIALS:

**1. Test material** Dithiopyr technical

**Description:** Solid

**Lot No./Batch No.:** 1A08164B01

**Purity:** 94.9%

**Stability of compound under test conditions:** The 96-hour measured concentrations ranged from 43 to 66% of their initial measured counterparts. See reviewer comments.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

**Storage conditions of test chemicals:** Ambient temperature in the dark.

### Physicochemical properties of Dithiopyr.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	

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Parameter	Values	Comments
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

## 2. Test organism:

**Name:** Freshwater diatom, *Navicula pelliculosa*

*EPA requires a nonvascular species: For tier I testing, only one species, *S. capricornutum*, to be tested; for tier II testing, *S. costatum*, *A. flos-aquae*, *S. capricornutum*, and a freshwater diatom is tested.*

*OECD suggests the following species are considered suitable: *S. capricornutum*, *S. subspicatus*, and *C. vulgaris*. If other species are used, the strain should be reported*

**Strain:** Not reported  
**Source:** In-house cultures originally obtained from the Canadian Phycological Culture Center (CPCC).  
**Age of inoculum:** 3 days  
**Method of cultivation:** Cultured in freshwater AAP Media under continuous fluorescent light at  $4300 \pm 10\%$  lux at  $24 \pm 2^\circ\text{C}$  and shaken at 100 rpm.

## B. STUDY DESIGN:

### 1. Experimental Conditions

- Range-finding study: A preliminary range finding was conducted at nominal concentrations of 0.10, 1.0, 10 and 100  $\mu\text{g ai/L}$  and at 96 hours yielded inhibitions of 18, -6, -4 and 99%, respectively.
- b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks
<i>Criteria</i>		
Acclimation period:	Continuously cultured in-house	
Culturing media and conditions:	Same as test	

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Parameter	Details	Remarks
		Criteria
(same as test or not)		<i>EPA recommends two week acclimation period.</i>
Health: (any mortality observed)	Actively growing for at least two weeks.	<i>OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.</i>
Test system Static/static renewal	Static	
Renewal rate for static renewal	N/A	<i>EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7-day test, 3-4 renewals for the 14 day test).</i>
Incubation facility	Temperature-controlled environmental chamber with a shaker table	
Duration of the test	96 hours	<i>EPA requires: 96-120 hours OECD: 72 hours</i>
Test vessel Material: (glass/stainless steel) Size: Fill volume:	Glass 250 mL 100 mL	Erlenmeyer plugged with sterile foam stoppers.  <i>OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.</i>
Details of Freshwater AAP		

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Parameter	Details	Remarks
		Criteria
medium pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	7.4 to 7.5 7.4 to 9.5 Na <sub>2</sub> EDTA·2H <sub>2</sub> O NaHCO <sub>3</sub> N/A	<i>OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.</i>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	<i>EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91 and D 3978-80 (reapproved 1987).</i>
Dilution water source/type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Purified (NANOpure water) Not reported N/A Filter sterilized Not reported Not reported Not reported Below detection limits Not reported	<i>EPA pH: <i>Skeletonema costatum</i> = ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.</i>  <i>OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.</i>

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Parameter	Details	Remarks
		Criteria
Indicate how the test material is added to the medium (added directly or used stock solution)	A primary stock solution was prepared by dissolving 0.0105 g of the test substance in 100 mL acetonitrile to achieve a nominal concentration of 100 µg ai/mL. Four additional stock solutions were prepared by adding 500 µL of each stock to a 500 mL glass volumetric flask, evaporating the solvent under a stream of nitrogen After the solvent evaporated freshwater algal medium was added.	
Aeration or agitation	Constantly shaken at rate of 100 rpm	
Initial cells density	5,000 cells/mL	<p><i>EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.</i></p> <p><i>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i>. When other species are used the biomass should be comparable.</i></p>

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Parameter	Details	Remarks
		<i>Criteria</i>
Number of replicates Control: Solvent control: Treatments:	8 N/A 4	<p><i>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <i>Navicula</i> sp. tests should be conducted with four replicate.</i></p>
		<p><i>OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.</i></p>
Test concentrations Nominal:	0 (negative control), 4.3, 9.4, 21, 45, and 100 µg/L	
Geometric mean measured:	<0.313 (<LOQ, negative control), 2.7, 5.8, 14, 29, and 65 µg ai/L	<p><i>EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.</i></p>
Initial measured:	<0.313 (<LOQ, negative control), 3.96, 8.26, 20.8, 40.3, and 79.5 µg ai/L	<p><i>OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.</i></p>
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	Test concentrations were measured at 0 and 96 hours using HPLC analysis.	

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Parameter	Details	Remarks
		Criteria
Test conditions Temperature: Photoperiod: Light intensity and quality:	23.24 to 23.42°C Continuous 4020-4660 lux fluorescent light	<p>EPA temperature: <i>Skeletonema</i>: 20EC, Others: 24-25EC; EPA photoperiod: <i>S. costatum</i> 14 hr light/ 10 hr dark. Others: Continuous; EPA light: <i>Anabaena</i>: 2.0 Klux (<math>\pm 15\%</math>), Others: 4 - 5 Klux (<math>\pm 15\%</math>)</p> <p>OECD recommended the temperature in the range of 21 to 25°C maintained at <math>\pm 2^\circ\text{C}</math> and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.</p>
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	N/A	

## 2. Observations:

**Table 2: Observation parameters**

Parameters	Details	Remarks
		Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell density Yield Growth rate Area under the curve (AUC)	<p>EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.</p>

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Parameters	Details	Remarks
		Criteria
Measurement technique for cell density and other end points	Cell density was determined using a light microscope and hemocytometer (Improved Neubauer). Yield was calculated as cell density at test termination minus test initiation. Growth rate was calculated from cell density using a logarithmic growth equation. AUC was calculated as the area under the cell density growth curve.	<i>EPA recommends the measurement technique of cell counts or chlorophyll a</i> <i>OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).</i>
Observation intervals	Every 24 hours	<i>EPA and OECD: every 24 hours.</i>
Other observations, if any	Cells were observed daily for health.	
Indicate whether there was an exponential growth in the control	Yes, after 96 hours, the mean cell density of the negative control increased 200-fold.	<i>EPA requires control cell count at termination to be <math>\geq 2X</math> initial count or by a factor of at least 16 during the test.</i> <i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i>
Were raw data included?	Yes, raw cell density data were provided.	

## II. RESULTS and DISCUSSION:

### A. INHIBITORY EFFECTS:

After 96 hours, the mean cell density of the negative control was  $265.23 \times 10^4$  cells/mL, yielding inhibitions relative to the negative control of 1.1, -17.5, -23.5, 6.9 and 98.8% for the initial measured exposure concentrations of 3.96, 8.26, 20.8, 40.3, and 79.5 µg ai/L, respectively. The study author did not assess cell density.

The mean 0-96-hour yield of the negative control was  $264.73 \times 10^4$  cells/mL, yielding inhibitions relative to the negative control of 1.1, -17.5, -23.6, 6.9, and 99.0% for the initial measured exposure concentrations of 3.96, 8.26, 20.8, 40.3, and 79.5 µg ai/L, respectively. The study author reported inhibitions of 1, -18, -24, 7 and 99% for the geometric mean measured exposure concentrations of 2.7, 5.8, 14, 29, and 65 µg ai/L, respectively. The NOAEC and IC<sub>50</sub> values reported by the study author based on yield were 29 and 37 µg ai/L, respectively, in terms of geometric mean measured exposure concentrations.

The mean 0-96-hour growth rate of the negative control was 0.0653/hour, yielding inhibitions relative to the negative control of 0.1, -2.7, -3.5, 1.1, and 72.6% for the initial measured exposure concentrations of 3.96, 8.26, 20.8, 40.3, and 79.5 µg ai/L, respectively. The study author reported inhibitions of 0, -3, -3, 1, and 73% for the

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geometric mean measured exposure concentrations of 2.7, 5.8, 14, 29, and 65 µg ai/L, respectively. The NOAEC and IC<sub>50</sub> values reported by the study author based on growth rate were 29 and 54 µg ai/L, respectively, in terms of geometric mean measured exposure concentrations.

The mean 0-96 hour AUC value of the negative control was 7555, yielding inhibitions relative to the negative control of -3.9, -17.6, -24.9, 22.2, and 98.2% for the initial measured exposure concentrations of 3.96, 8.26, 20.8, 40.3, and 79.5 µg ai/L, respectively. The study author reported inhibitions of -4, -18, -25, 22 and 98% for the geometric mean measured exposure concentrations of 2.7, 5.8, 14, 29, and 65 µg ai/L, respectively. The NOAEC and IC<sub>50</sub> values reported by the study author based on AUC were 14 and 34 µg ai/L, respectively, in terms of mean measured exposure concentrations.

No morphological abnormalities were noted. There were increases in pH during the test. There were no compound-related phytotoxic effects.

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**Table 3: Measured concentrations of Dithiopyr (freshwater diatom, *Navicula pelliculosa*)**

Treatment Initial measured (geometric mean measured) and [nominal] concentrations µg ai/L	Measured Concentrations	
	0 hours (Day 0)	96 hours (Day 4)
Negative control	<LOQ	<LOQ
3.96 (2.7) [4.3]	3.96	1.83
8.26 (5.8) [9.4]	8.26	4.07
20.8 (14) [21]	20.8	8.85
40.3 (29) [45]	40.3	20.2
79.5 (65) [100]	79.5	52.6

**Table 4: Effect of Dithiopyr on algal growth (freshwater diatom, *Navicula pelliculosa*)**

Treatment Initial measured (geometric mean measured) and [nominal] concentrations µg ai/L	Initial cell density (x10 <sup>4</sup> cells/mL)	Cell density (x10 <sup>4</sup> cells/mL) at			
		48 hours	72 hours	96 hours	
				cell count	% inhibition <sup>a</sup>
Negative control	0.5	36.36	142.26	265.23	N/A
3.96 (2.7) [4.3]	0.5	43.48	148.43	262.43	1.1
8.26 (5.8) [9.4]	0.5	41.70	169.13	311.65	-17.5
20.8 (14) [21]	0.5	39.03	186.95	327.63	-23.5
40.3 (29) [45]	0.5	17.50	102.33	246.93	6.9
79.5 (65) [100]	0.5	1.98	2.30	3.23	98.8
Reference chemical (if used)	N/A				

<sup>a</sup> Calculated by the reviewer relative to the negative control.

**Table 5: Effect of Dithiopyro algal growth (freshwater diatom, *Navicula pelliculosa*)**

Treatment Initial measured (geometric mean measured) and [nominal] concentrations µg ai/L	Initial cell density (x10 <sup>4</sup> cells/mL)	Mean growth rate (hour <sup>-1</sup> )		Mean area under the curve (AUC)		Mean yield (based on cell density; x10 <sup>4</sup> cells/mL)	
		0-96 hours	% inhibition <sup>a</sup>	0-96 hours	% inhibition <sup>a</sup>	0-96 hours	% inhibition <sup>a</sup>

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Negative control	0.5	0.0653	N/A	7555	N/A	264.73	N/A
3.96 (2.7) [4.3]	0.5	0.0652	0.1	7847	-3.9	261.93	1.1
8.26 (5.8) [9.4]	0.5	0.0670	-2.7	8885	-17.6	311.15	-17.5
20.8 (14) [21]	0.5	0.0675	-3.5	9438	-24.9	327.13	-23.6
40.3 (29) [45]	0.5	0.0646	1.1	5877	22.2	246.43	6.9
79.5 (65) [100]	0.5	0.0179*	72.6	134*	98.2	2.73*	99.0

<sup>a</sup> Calculated by the reviewer relative to the negative control.

\* Determined by the study author to be statistically reduced ( $p \leq 0.05$ , Dunnett's test) when compared to the negative control.

**Table 6: Statistical endpoint values.\* (calculated by the study author based on geometric mean measured concentrations)**

Statistical endpoint	Cell density	Yield	Growth rate	Area under the curve (AUC)
NOAEC ( $\mu\text{g ai/L}$ )	Not calculated	29	29	14
LOAEC ( $\mu\text{g ai/L}$ )	Not calculated	Not calculated	Not calculated	Not calculated
IC <sub>50</sub> or EC <sub>50</sub> ( $\mu\text{g ai/L}$ ) (95% C.I.)	Not calculated	Not calculated	Not calculated	Not calculated
IC <sub>20</sub> or EC <sub>20</sub> ( $\mu\text{g ai/L}$ ) (95% C.I.)	Not calculated	Not calculated	Not calculated	Not calculated
IC <sub>50</sub> or EC <sub>50</sub> ( $\mu\text{g ai/L}$ ) (95% C.I.)	Not calculated	37 (34 to 41)	54 (48 to 60)	34 (32 to 37)
Reference chemical, if used	N/A			

\* Do not use this table, if the study was deemed unacceptable.

NA = Not applicable.

### B. REPORTED STATISTICS:

The study author statistically analyzed the endpoints for mean growth rates and yield using The SAS System for Windows Version 9.4. EC<sub>50</sub> values were calculated using nonlinear regression. EC<sub>50</sub> values were calculated using non-linear regression. The data were assessed for normality and homogeneity of variance using Shapiro-Wilk's and Levene's tests, respectively. If the data passed these tests, Dunnett's test was used to determine the NOEC. If the data did not pass, the NOEC was determined using Jonckheere-Terpstra Step-Down Trend test. All analyses were based on geometric mean measured concentrations.

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## C. VERIFICATION OF STATISTICAL RESULTS:

**Statistical Method:** The reviewer statistically analyzed the 96-hour endpoints for yield, growth rate, and AUC using CETIS version 1.8.7.12 statistical software using backend database settings implemented by EFED on 10/20/15. The data were assessed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's tests, respectively. Yield and area under the curve (AUC) data met both assumptions of normality and homogeneity of variance and were therefore analyzed using Dunnett's test. Growth rate data had non-normal distribution and unequal variances and were analyzed using Mann-Whitney test. The IC<sub>x</sub> values were calculated using Bruce-Versteeg regression. The IC<sub>50</sub> value for yield was not calculable. Toxicity values are reported in terms of initial measured exposure concentrations.

### *Yield*

IC <sub>05</sub> :	35.8 µg ai/L	95% C.I.: 0 to 40.3 µg ai/L
IC <sub>50</sub> :	Not calculable	95% C.I.: N/A
NOAEC:	40.3 µg ai/L	
Probit Slope:	N/A	

### *Growth rate*

IC <sub>05</sub> :	44 µg ai/L	95% C.I.: 0 to 49.4 µg ai/L
IC <sub>50</sub> :	68 µg ai/L	95% C.I.: 64.6 to 71.7 µg ai/L
NOAEC:	40.3 µg ai/L	
Probit Slope:	N/A	

### *Area under the curve*

IC <sub>05</sub> :	31.2 µg ai/L	95% C.I.: 0 to 39.5 µg ai/L
IC <sub>50</sub> :	45.5 µg ai/L	95% C.I.: 32.1 to 64.5 µg ai/L
NOAEC:	20.8 µg ai/L	
Probit Slope:	N/A	

## D. STUDY DEFICIENCIES:

1. The strain of the test organism was not reported.
2. The test substance was unstable under test conditions for the test solutions, with 96-hour measured concentrations ranging from 43 to 66% of their initial measured counterparts.
3. The pH, TOC, particulate matter, and metals, and chlorine concentrations of the dilution water were not reported.
4. The physico-chemical properties of the test material were not reported.
5. The initial cell density ( $0.5 \times 10^4$  cells/mL) was lower than recommended ( $1.0 \times 10^4$  cells/mL).

## E. REVIEWER'S COMMENTS:

The reviewer's values were in general agreement with those of the study author with the exception that the reviewer reported results based on the initial measured concentrations, whereas the study author used the geometric mean-measured concentrations. The reviewer's results are reported in the Executive Summary and Conclusions sections of this report.

As part of the stock solution preparation, the test material was dissolved in acetonitrile, which was evaporated off. Saltwater algal medium was added to complete the stock solutions. The study authors indicated that this procedure was carried out to increase accuracy associated with test concentrations and in order to avoid the use of a solvent in the test. Similar approaches have been attempted in sediment toxicity studies with invertebrates. There is uncertainty associated with the addition of acetonitrile to the stock solutions, as this was not incorporated

## Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Navicula pelliculosa*

PMRA Submission Number {.....}

EPA MRID Number 49760109

into the controls. This approach assumes that acetonitrile is completely evaporated prior to addition of the saltwater medium and so algae are not exposed to acetonitrile.

Since this compound has a Koc ranging 1396-3049, and the chemical is stable due to hydrolysis, photolysis half-lives are on the order of weeks and aerobic soil metabolism half-lives are >1 year (based on information in the registration review problem formulation), it is the reviewer's opinion that the lack of stability of the compound in the test vessels is due to sorption, not degradation.

The in-life phase of the definitive test was conducted from 11 to 15 May 2015.

The coefficient of variation (CV) of control yield was 11.4%, which meets the guideline requirement of yield CV<15%. The CV of control growth rate was 1.87%, which meets the guideline requirement of growth rate CV<15%.

### F. CONCLUSIONS:

This study is scientifically sound and is classified as acceptable. After 96 hours, the most sensitive endpoint was area under the curve (AUC) with NOAEC and IC<sub>50</sub> values of 20.8 and 45.5 µg ai/L, respectively, based on initial measured concentrations.

#### *Yield*

IC <sub>05</sub> :	35.8 µg ai/L	95% C.I.: 0 to 40.3 µg ai/L
IC <sub>50</sub> :	Not calculable	95% C.I.: N/A
NOAEC:	40.3 µg ai/L	
Probit Slope:	N/A	

#### *Growth rate*

IC <sub>05</sub> :	44 µg ai/L	95% C.I.: 0 to 49.4 µg ai/L
IC <sub>50</sub> :	68 µg ai/L	95% C.I.: 64.6 to 71.7 µg ai/L
NOAEC:	40.3 µg ai/L	
Probit Slope:	N/A	

#### *Area under the curve*

IC <sub>05</sub> :	31.2 µg ai/L	95% C.I.: 0 to 39.5 µg ai/L
IC <sub>50</sub> :	45.5 µg ai/L	95% C.I.: 32.1 to 64.5 µg ai/L
NOAEC:	20.8 µg ai/L	
Probit Slope:	N/A	

Endpoint(s) Effected: Yield, growth rate, and area under the curve.

### III. REFERENCES:

Organization for Economic Cooperation and Development. 2006. OECD Guidelines for Testing of Chemicals, Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. Adopted 23 March 2006.

U.S. Environmental Protection Agency. 2012. Series 850-Ecological Effects Test Guidelines, OCSPP Number 850.4500: *Algal Toxicity*. American Society for Testing and Materials. 2004.

ASTM Standard Guide E1218-04. *Standard Guide for Conducting Static Toxicity Tests with Microalgae*.

## **Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Navicula pelliculosa***

PMRA Submission Number {.....}

EPA MRID Number 49760109

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The SAS System for Windows. 2002-2014. Version 9.4. SAS Institute, Inc., Cary, North Carolina.

Bruce, Robert D. and Donald J. Versteeg. 1992. A Statistical Procedure for Modeling Continuous Toxicity Data. Environmental Toxicology and Chemistry. II: 1485-1494.

## OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	07-4655-5436	Endpoint:	96h AUC	CETIS Version:	CETISv1.8.7
Analyzed:	24 Mar-17 11:12	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes
Batch ID:	01-6116-4317	Test Type:	Algal Cell Growth (96-h)	Analyst:	
Start Date:	11 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent:	Algal Culture Media
Ending Date:		Species:	Navicula pelliculosa	Brine:	
Duration:	NA	Source:	Canadian Phycological Culture Centre	Age:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	13.1%	21	40	28.98	

## Dunnett Multiple Comparison Test

Control	vs	C- $\mu$ g ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision( $\alpha$ :5%)
Negative Control	4		-0.718	2.43	987	10	0.9830	CDF	Non-Significant Effect
	8.3		-3.27	2.43	987	10	1.0000	CDF	Non-Significant Effect
	21		-4.63	2.43	987	10	1.0000	CDF	Non-Significant Effect
	40*		4.13	2.43	987	10	0.0010	CDF	Significant Effect
	80*		18.3	2.43	987	10	<0.0001	CDF	Significant Effect

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision( $\alpha$ :5%)
Between	235246800	47049360	5	107	<0.0001	Significant Effect
Error	9683252	440147.8	22			
Total	244930100		27			

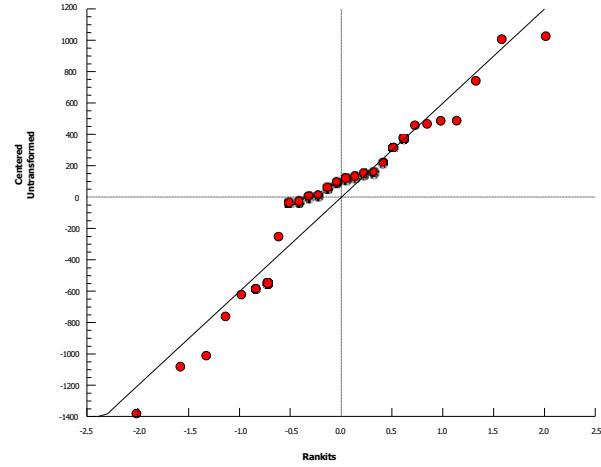
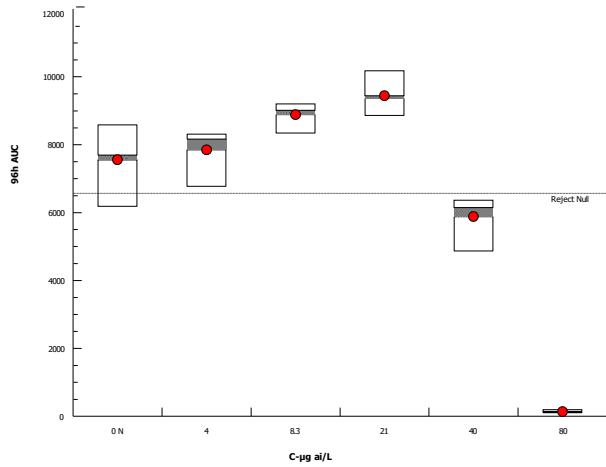
## Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision( $\alpha$ :1%)
Variances	Bartlett Equality of Variance	14.2	15.1	0.0147	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.951	0.897	0.2085	Normal Distribution

## 96h AUC Summary

C- $\mu$ g ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	7.55E+3	6.83E+3	8.28E+3	7680	6.17E+3	8.58E+3	3.05E+2	11.4%	0.0%
4		4	7.85E+3	6.68E+3	9.02E+3	8160	6.76E+3	8.31E+3	3.68E+2	9.38%	-3.86%
8.3		4	8.88E+3	8.27E+3	9.50E+3	9000	8.33E+3	9.20E+3	1.94E+2	4.38%	-17.6%
21		4	9.44E+3	8.54E+3	1.03E+4	9360	8.85E+3	1.02E+4	2.83E+2	6.0%	-24.9%
40		4	5.88E+3	4.78E+3	6.97E+3	6140	4.86E+3	6.36E+3	3.44E+2	11.7%	22.2%
80		4	1.34E+2	6.31E+1	2.05E+2	122	9.72E+1	1.96E+2	2.23E+1	33.3%	98.2%

## Graphics



## OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	02-3649-8417	Endpoint:	96h Cell Density	CETIS Version:	CETISv1.8.7
Analyzed:	24 Mar-17 11:12	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes
Batch ID:	01-6116-4317	Test Type:	Algal Cell Growth (96-h)	Analyst:	
Start Date:	11 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent:	Algal Culture Media
Ending Date:		Species:	Navicula pelliculosa	Brine:	
Duration:	NA	Source:	Canadian Phycological Culture Centre	Age:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	12.4%	40	80	56.57	

## Dunnett Multiple Comparison Test

Control	vs	C- $\mu$ g ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision( $\alpha$ :5%)
Negative Control	4		0.207	2.43	32.8	10	0.8256	CDF	Non-Significant Effect
	8.3		-3.44	2.43	32.8	10	1.0000	CDF	Non-Significant Effect
	21		-4.62	2.43	32.8	10	1.0000	CDF	Non-Significant Effect
	40		1.36	2.43	32.8	10	0.3123	CDF	Non-Significant Effect
	80*		19.4	2.43	32.8	10	<0.0001	CDF	Significant Effect

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision( $\alpha$ :5%)
Between	282787.8	56557.55	5	116	<0.0001	Significant Effect
Error	10686.75	485.7616	22			
Total	293474.5		27			

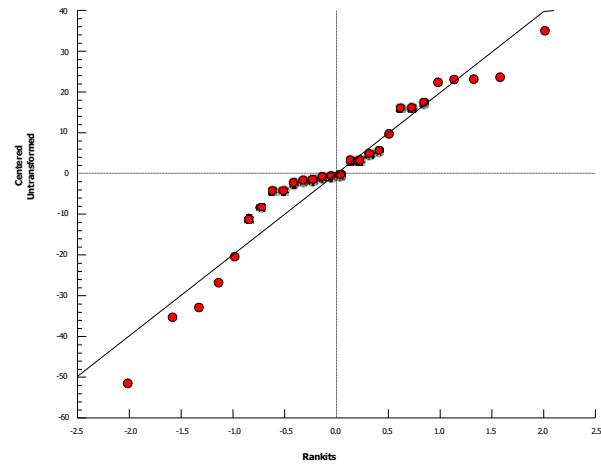
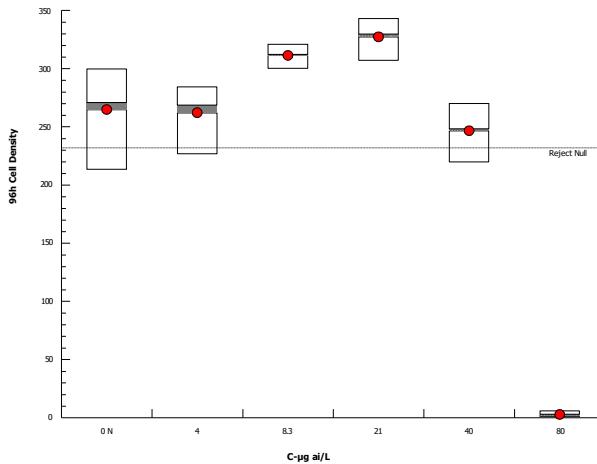
## Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision( $\alpha$ :1%)
Variances	Bartlett Equality of Variance	14.8	15.1	0.0113	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.949	0.897	0.1843	Normal Distribution

## 96h Cell Density Summary

C- $\mu$ g ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	265	240	290	271	213	300	10.7	11.4%	0.0%
4		4	262	220	304	268	227	284	13.1	10.0%	1.06%
8.3		4	311	297	325	312	300	321	4.4	2.83%	-17.5%
21		4	327	303	351	329	307	343	7.61	4.65%	-23.6%
40		4	246	213	280	248	220	270	10.5	8.49%	6.91%
80		4	2.72	-0.728	6.18	2	1	5.9	1.09	79.6%	99.0%

## Graphics



## OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	13-0525-2850	Endpoint:	96h Growth Rate	CETIS Version:	CETISv1.8.7
Analyzed:	24 Mar-17 11:12	Analysis:	Nonparametric-Two Sample	Official Results:	Yes
Batch ID:	01-6116-4317	Test Type:	Algal Cell Growth (96-h)	Analyst:	
Start Date:	11 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent:	Algal Culture Media
Ending Date:		Species:	Navicula pelliculosa	Brine:	
Duration:	NA	Source:	Canadian Phycological Culture Centre	Age:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	4.26%	40	80	56.57	

## Mann-Whitney U Two-Sample Test

Control	vs	C- $\mu$ g ai/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision( $\alpha$ :5%)
Negative Control		4	19	NA	0	10	0.3232	Exact	Non-Significant Effect
		8.3	0.5	NA	1	10	0.9980	Exact	Non-Significant Effect
		21	0	NA	0	10	1.0000	Exact	Non-Significant Effect
		40	23	NA	0	10	0.1293	Exact	Non-Significant Effect
		80*	32	NA	0	10	0.0020	Exact	Significant Effect

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision( $\alpha$ :5%)
Between	0.007906073	0.001581215	5	251	<0.0001	Significant Effect
Error	0.0001383713	6.289602E-06	22			
Total	0.008044444		27			

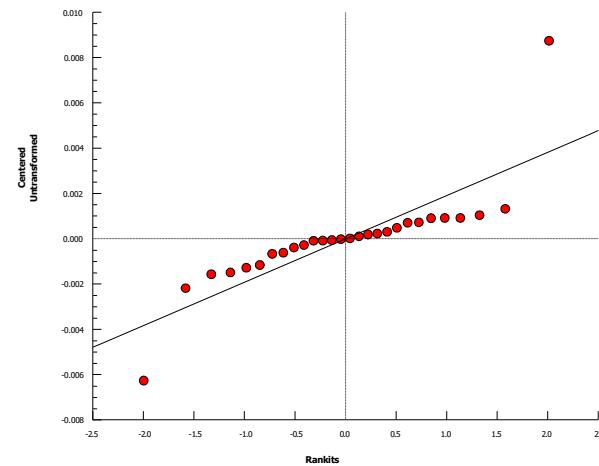
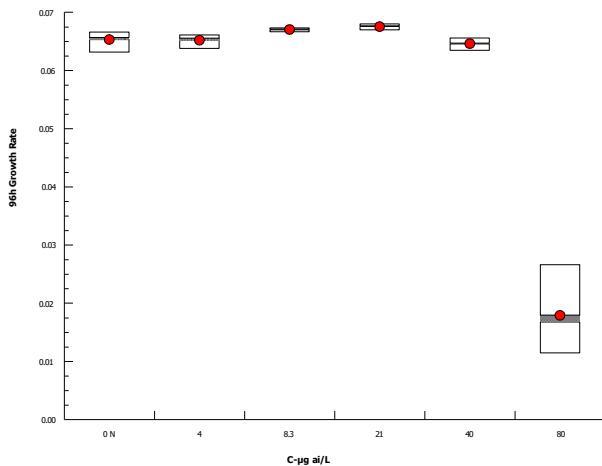
## Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision( $\alpha$ :1%)
Variances	Bartlett Equality of Variance	34.5	15.1	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.72	0.897	<0.0001	Non-normal Distribution

## 96h Growth Rate Summary

C- $\mu$ g ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	0.0653	0.0643	0.0663	0.0656	0.0631	0.0666	0.000432	1.87%	0.0%
4		4	0.0652	0.0635	0.0669	0.0655	0.0637	0.0661	0.000545	1.67%	0.13%
8.3		4	0.067	0.0665	0.0675	0.0671	0.0666	0.0673	0.000147	0.44%	-2.62%
21		4	0.0675	0.0668	0.0683	0.0676	0.0669	0.068	0.000232	0.69%	-3.43%
40		4	0.0646	0.0631	0.066	0.0646	0.0634	0.0656	0.000455	1.41%	1.09%
80		4	0.0179	0.00777	0.028	0.0168	0.0114	0.0266	0.00318	35.5%	72.6%

## Graphics



# CETIS Analytical Report

Report Date: 24 Mar-17 11:14 (p 1 of 6)  
 Test Code: 128994 49760109 | 10-0748-1929

## OCSPP 850.4500 Algal Toxicity

Wildlife International

<b>Analysis ID:</b> 03-2633-9845	<b>Endpoint:</b> 96h AUC	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 24 Mar-17 11:12	<b>Analysis:</b> Nonlinear Regression	<b>Official Results:</b> Yes
<b>Batch ID:</b> 01-6116-4317	<b>Test Type:</b> Algal Cell Growth (96-h)	<b>Analyst:</b>
<b>Start Date:</b> 11 May-15	<b>Protocol:</b> OCSPP 850.4500 Aquatic Plant (Algae)	<b>Diluent:</b> Algal Culture Media
<b>Ending Date:</b>	<b>Species:</b> Navicula pelliculosa	<b>Brine:</b>
<b>Duration:</b> NA	<b>Source:</b> Canadian Phycological Culture Centre	<b>Age:</b>

### Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

### Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
12	-204	414	417	0.9052	Yes	8.95	3.05	0.0005	Significant Lack of Fit

### Point Estimates

Level	µg ai/L	95% LCL	95% UCL
IC5	31.2	N/A	39.5
IC20	37.5	28.7	42.4
IC50	45.5	32.1	64.5

### Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	8260	208	7850	8660	39.7	<0.0001	Significant Parameter
C	0.229	0.221	-0.205	0.663	1.04	0.3104	Non-Significant Parameter
D	45.5	5.83	34.1	56.9	7.81	<0.0001	Significant Parameter

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	2.23E+08	2.23E+08	1	260	<0.0001	Significant
Lack of Fit	11823440	3941146	3	8.95	0.0005	Significant
Pure Error	9683252	440147.8	22			
Residual	21506690	860267.5	25			

### Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	14.2	11.1	0.0147	Unequal Variances
	Mod Levene Equality of Variance	1.16	2.66	0.3620	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.959	0.926	0.3361	Normal Distribution
	Anderson-Darling A2 Normality	0.675	2.49	0.0781	Normal Distribution

### 96h AUC Summary

C-µg ai/L	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	7.55E+3	6.17E+3	8.58E+3	3.05E+2	8.63E+2	11.4%	0.0%
4		4	7.85E+3	6.76E+3	8.31E+3	3.68E+2	7.36E+2	9.38%	-3.86%
8.3		4	8.88E+3	8.33E+3	9.20E+3	1.94E+2	3.89E+2	4.38%	-17.6%
21		4	9.44E+3	8.85E+3	1.02E+4	2.83E+2	5.67E+2	6.0%	-24.9%
40		4	5.88E+3	4.86E+3	6.36E+3	3.44E+2	6.89E+2	11.7%	22.2%
80		4	1.34E+2	9.72E+1	1.96E+2	2.23E+1	4.46E+1	33.3%	98.2%

## OCSPP 850.4500 Algal Toxicity

Wildlife International

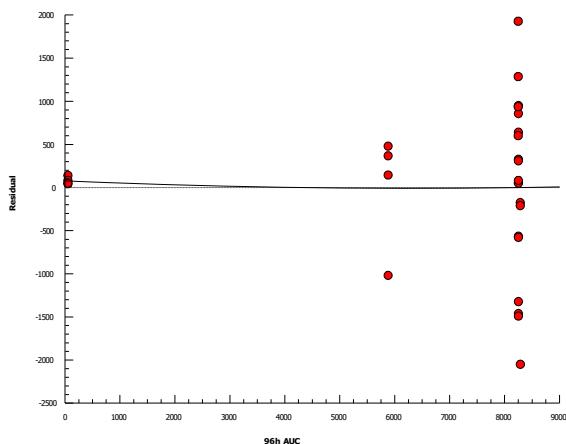
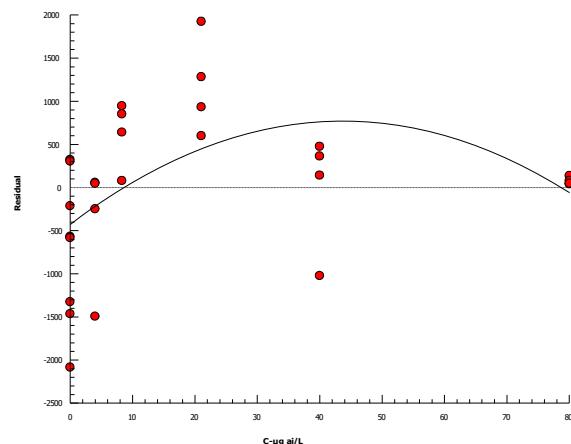
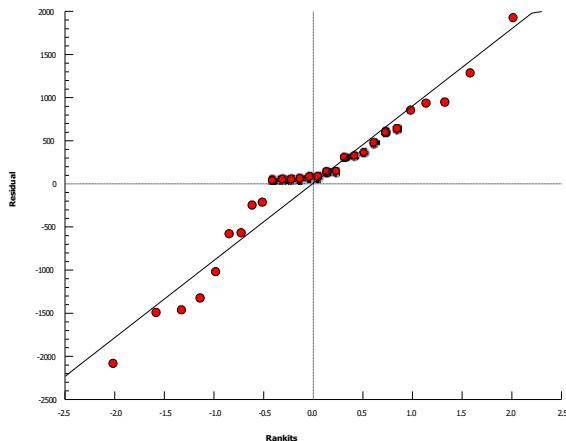
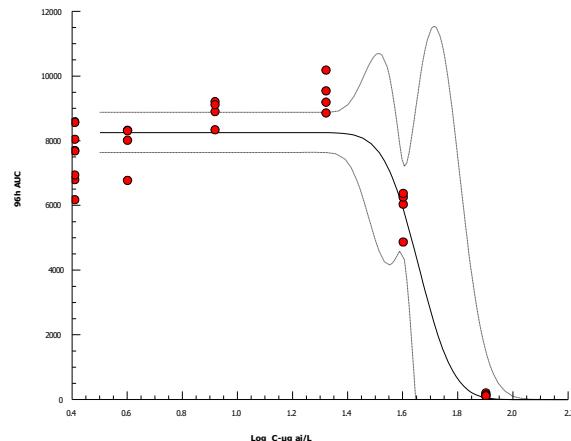
Analysis ID: 03-2633-9845  
 Analyzed: 24 Mar-17 11:12

Endpoint: 96h AUC  
 Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7  
 Official Results: Yes

## Graphics

3P Cumulative Log-Normal EV [Y=A\*(1- Φ(log(X/D)/C))]



**CETIS Analytical Report**

**Report Date:** 24 Mar-17 11:14 (p 3 of 6)  
**Test Code:** 128994 49760109 | 10-0748-1929

**OCSPP 850.4500 Algal Toxicity****Wildlife International**

<b>Analysis ID:</b> 01-0673-2768	<b>Endpoint:</b> 96h Cell Density	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 24 Mar-17 11:12	<b>Analysis:</b> Nonlinear Regression	<b>Official Results:</b> Yes
<b>Batch ID:</b> 01-6116-4317	<b>Test Type:</b> Algal Cell Growth (96-h)	<b>Analyst:</b>
<b>Start Date:</b> 11 May-15	<b>Protocol:</b> OCSPP 850.4500 Aquatic Plant (Algae)	<b>Diluent:</b> Algal Culture Media
<b>Ending Date:</b>	<b>Species:</b> Navicula pelliculosa	<b>Brine:</b>
<b>Duration:</b> NA	<b>Source:</b> Canadian Phycological Culture Centre	<b>Age:</b>

**Non-Linear Regression Options**

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

**Regression Summary**

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
5	-110	226	229	0.9046	Yes	10.5	3.05	0.0002	Significant Lack of Fit

**Point Estimates**

Level	µg ai/L	95% LCL	95% UCL
IC5	35.8	N/A	40.3
IC20	42	35.2	46.3
IC50	49.8	N/A	N/A

**Regression Parameters**

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	286	7.2	272	300	39.7	<0.0001	Significant Parameter
C	0.201	0.133	-0.0583	0.461	1.52	0.1411	Non-Significant Parameter
D	49.8	7.38	35.3	64.3	6.75	<0.0001	Significant Parameter

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	267552.4	267552.4	1	258	<0.0001	Significant
Lack of Fit	15235.35	5078.449	3	10.5	0.0002	Significant
Pure Error	10686.75	485.7616	22			
Residual	25922.1	1036.884	25			

**Residual Analysis**

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	14.8	11.1	0.0113	Unequal Variances
	Mod Levene Equality of Variance	2.45	2.66	0.0659	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.961	0.926	0.3776	Normal Distribution
	Anderson-Darling A2 Normality	0.536	2.49	0.1732	Normal Distribution

**96h Cell Density Summary**

C-µg ai/L	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	265	213	300	10.7	30.2	11.4%	0.0%
4		4	262	227	284	13.1	26.2	10.0%	1.06%
8.3		4	311	300	321	4.4	8.79	2.83%	-17.5%
21		4	327	307	343	7.61	15.2	4.65%	-23.6%
40		4	246	220	270	10.5	20.9	8.49%	6.91%
80		4	2.72	1	5.9	1.09	2.17	79.6%	99.0%

# CETIS Analytical Report

Report Date: 24 Mar-17 11:14 (p 4 of 6)  
Test Code: 128994 49760109 | 10-0748-1929

## OCSPP 850.4500 Algal Toxicity

Wildlife International

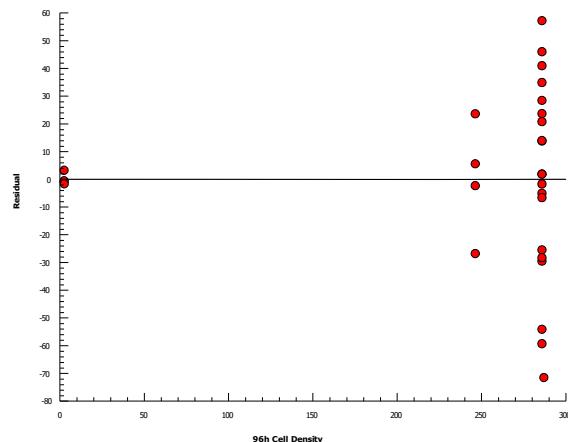
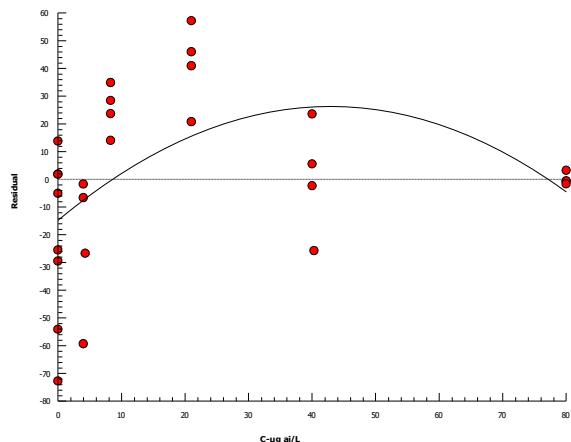
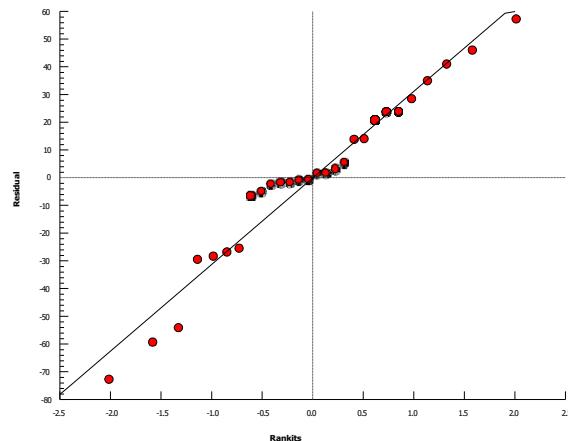
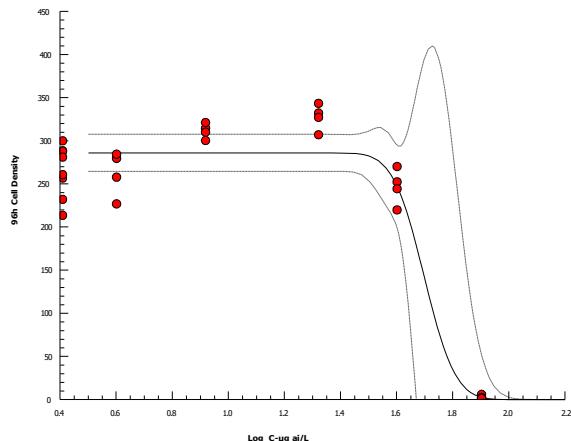
Analysis ID: 01-0673-2768  
Analyzed: 24 Mar-17 11:12

Endpoint: 96h Cell Density  
Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7  
Official Results: Yes

### Graphics

3P Cumulative Log-Normal EV [Y=A\*(1- Φ(log(X/D)/C))]



**CETIS Analytical Report**

**Report Date:** 24 Mar-17 11:14 (p 5 of 6)  
**Test Code:** 128994 49760109 | 10-0748-1929

**OCSPP 850.4500 Algal Toxicity****Wildlife International**

<b>Analysis ID:</b> 21-3852-7260	<b>Endpoint:</b> 96h Growth Rate	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 24 Mar-17 11:12	<b>Analysis:</b> Nonlinear Regression	<b>Official Results:</b> Yes
<b>Batch ID:</b> 01-6116-4317	<b>Test Type:</b> Algal Cell Growth (96-h)	<b>Analyst:</b>
<b>Start Date:</b> 11 May-15	<b>Protocol:</b> OCSPP 850.4500 Aquatic Plant (Algae)	<b>Diluent:</b> Algal Culture Media
<b>Ending Date:</b>	<b>Species:</b> Navicula pelliculosa	<b>Brine:</b>
<b>Duration:</b> NA	<b>Source:</b> Canadian Phycological Culture Centre	<b>Age:</b>

**Non-Linear Regression Options**

<b>Model Function</b>	<b>X Transform</b>	<b>Y Transform</b>	<b>Weighting Function</b>	<b>PTBS Function</b>
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

**Regression Summary**

<b>Iters</b>	<b>Log LL</b>	<b>AICc</b>	<b>BIC</b>	<b>Adj R2</b>	<b>Optimize</b>	<b>F Stat</b>	<b>Critical</b>	<b>P-Value</b>	<b>Decision(α:5%)</b>
5	155	-303	-300	0.9788	Yes	1.05	3.05	0.3893	Non-Significant Lack of Fit

**Point Estimates**

<b>Level</b>	<b>µg ai/L</b>	<b>95% LCL</b>	<b>95% UCL</b>
IC5	44	N/A	49.4
IC20	54.4	47.3	59.9
IC50	68	64.6	71.7

**Regression Parameters**

<b>Parameter</b>	<b>Estimate</b>	<b>Std Error</b>	<b>95% LCL</b>	<b>95% UCL</b>	<b>t Stat</b>	<b>P-Value</b>	<b>Decision(α:5%)</b>
A	0.0661	0.000563	0.065	0.0672	117	<0.0001	Significant Parameter
C	0.265	0.0396	0.187	0.342	6.7	<0.0001	Significant Parameter
D	68	1.86	64.4	71.7	36.6	<0.0001	Significant Parameter

**ANOVA Table**

<b>Source</b>	<b>Sum Squares</b>	<b>Mean Square</b>	<b>DF</b>	<b>F Stat</b>	<b>P-Value</b>	<b>Decision(α:5%)</b>
Model	0.007886	0.007886	1	1250	<0.0001	Significant
Lack of Fit	1.99E-05	6.62E-06	3	1.05	0.3893	Non-Significant
Pure Error	0.000138	6.29E-06	22			
Residual	0.000158	6.33E-06	25			

**Residual Analysis**

<b>Attribute</b>	<b>Method</b>	<b>Test Stat</b>	<b>Critical</b>	<b>P-Value</b>	<b>Decision(α:5%)</b>
Variances	Mod Levene Equality of Variance	2.68	2.66	0.0488	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.828	0.926	0.0004	Non-normal Distribution
	Anderson-Darling A2 Normality	1.62	2.49	<0.0001	Non-normal Distribution

**96h Growth Rate Summary**

<b>C-µg ai/L</b>	<b>Control Type</b>	<b>Count</b>	<b>Calculated Variate</b>						
			<b>Mean</b>	<b>Min</b>	<b>Max</b>	<b>Std Err</b>	<b>Std Dev</b>	<b>CV%</b>	<b>%Effect</b>
0	Negative Control	8	0.0653	0.0631	0.0666	0.000432	0.00122	1.87%	0.0%
4		4	0.0652	0.0637	0.0661	0.000545	0.00109	1.67%	0.13%
8.3		4	0.067	0.0666	0.0673	0.000147	0.000294	0.44%	-2.62%
21		4	0.0675	0.0669	0.068	0.000232	0.000464	0.69%	-3.43%
40		4	0.0646	0.0634	0.0656	0.000455	0.000911	1.41%	1.09%
80		4	0.0179	0.0114	0.0266	0.00318	0.00635	35.5%	72.6%

**CETIS Analytical Report**

Report Date: 24 Mar-17 11:14 (p 6 of 6)  
Test Code: 128994 49760109 | 10-0748-1929

**OCSPP 850.4500 Algal Toxicity****Wildlife International**

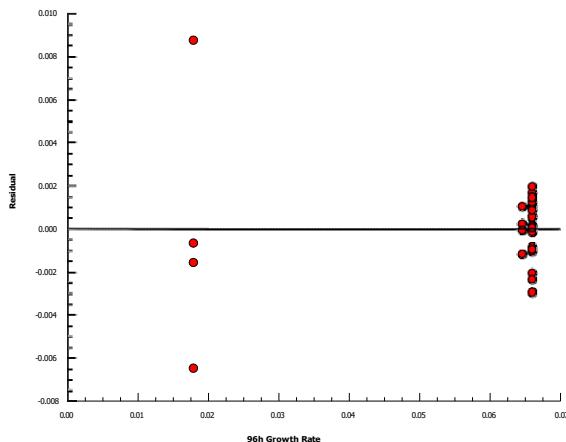
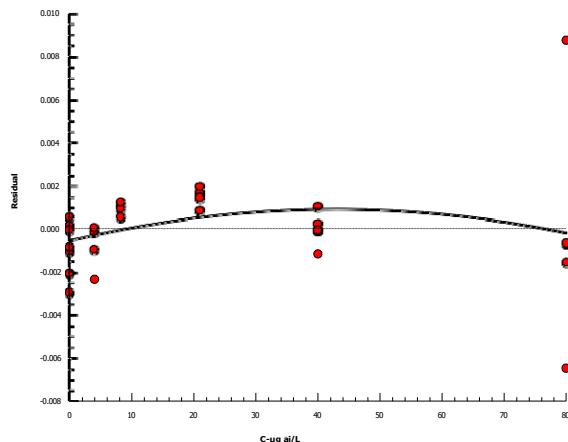
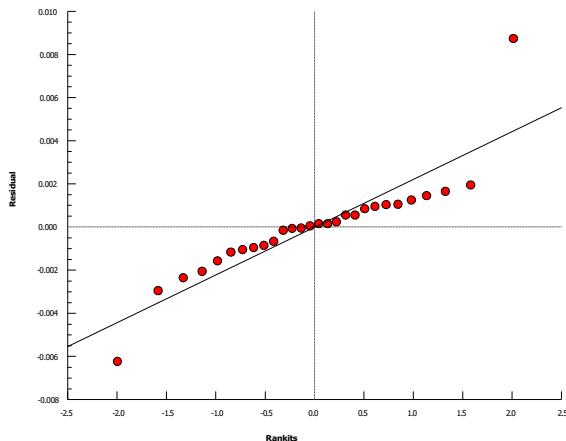
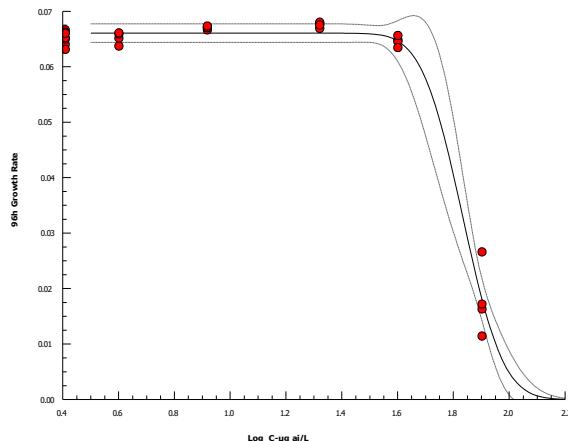
Analysis ID: 21-3852-7260  
Analyzed: 24 Mar-17 11:12

Endpoint: 96h Growth Rate  
Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7  
Official Results: Yes

**Graphics**

3P Cumulative Log-Normal EV [Y=A\*(1- Φ(log(X/D)/C))]



**CETIS Summary Report**

 Report Date: 24 Mar-17 11:15 (p 1 of 2)  
 Test Code: 128994 49760109 | 10-0748-1929

**OCSPP 850.4500 Algal Toxicity**
**Wildlife International**

<b>Batch ID:</b> 01-6116-4317	<b>Test Type:</b> Algal Cell Growth (96-h)	<b>Analyst:</b>
<b>Start Date:</b> 11 May-15	<b>Protocol:</b> OCSPP 850.4500 Aquatic Plant (Algae)	<b>Diluent:</b> Algal Culture Media
<b>Ending Date:</b>	<b>Species:</b> Navicula pelliculosa	<b>Brine:</b>
<b>Duration:</b> NA	<b>Source:</b> Canadian Phycological Culture Centre	<b>Age:</b>
<b>Sample ID:</b> 20-8590-0954	<b>Code:</b> 128994 49760109	<b>Client:</b> CDM Smith - D. Worcester
<b>Sample Date:</b> 11 May-15	<b>Material:</b> Dithiopyr	<b>Project:</b>
<b>Receive Date:</b>	<b>Source:</b> Dow AgroSciences	
<b>Sample Age:</b> NA	<b>Station:</b>	

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
07-4655-5436	96h AUC	21	40	28.98	13.1%		Dunnett Multiple Comparison Test
02-3649-8417	96h Cell Density	40	80	56.57	12.4%		Dunnett Multiple Comparison Test
13-0525-2850	96h Growth Rate	40	80	56.57	4.26%		Mann-Whitney U Two-Sample Test

**Point Estimate Summary**

Analysis ID	Endpoint	Level	µg ai/L	95% LCL	95% UCL	TU	Method
03-2633-9845	96h AUC	IC5	31.2	N/A	39.5		Nonlinear Regression
		IC20	37.5	28.7	42.4		
		IC50	45.5	32.1	64.5		
01-0673-2768	96h Cell Density	IC5	35.8	N/A	40.3		Nonlinear Regression
		IC20	42	35.2	46.3		
		IC50	49.8	N/A	N/A		
21-3852-7260	96h Growth Rate	IC5	44	N/A	49.4		Nonlinear Regression
		IC20	54.4	47.3	59.9		
		IC50	68	64.6	71.7		

**96h AUC Summary**

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	7550	6830	8280	6170	8580	305	863	11.4%	0.0%
4		4	7850	6680	9020	6760	8310	368	736	9.38%	-3.86%
8.3		4	8880	8270	9500	8330	9200	194	389	4.38%	-17.6%
21		4	9440	8540	10300	8850	10200	283	567	6.0%	-24.9%
40		4	5880	4780	6970	4860	6360	344	689	11.7%	22.2%
80		4	134	63.1	205	97.2	196	22.3	44.6	33.3%	98.2%

**96h Cell Density Summary**

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	265	240	290	213	300	10.7	30.2	11.4%	0.0%
4		4	262	220	304	227	284	13.1	26.2	10.0%	1.06%
8.3		4	311	297	325	300	321	4.4	8.79	2.83%	-17.5%
21		4	327	303	351	307	343	7.61	15.2	4.65%	-23.6%
40		4	246	213	280	220	270	10.5	20.9	8.49%	6.91%
80		4	2.73	-0.728	6.18	1	5.9	1.09	2.17	79.6%	99.0%

**96h Growth Rate Summary**

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	0.0653	0.0643	0.0663	0.0631	0.0666	0.000432	0.00122	1.87%	0.0%
4		4	0.0652	0.0635	0.0669	0.0637	0.0661	0.000545	0.00109	1.67%	0.13%
8.3		4	0.067	0.0665	0.0675	0.0666	0.0673	0.000147	0.000294	0.44%	-2.62%
21		4	0.0675	0.0668	0.0683	0.0669	0.068	0.000232	0.000465	0.69%	-3.43%
40		4	0.0646	0.0631	0.066	0.0634	0.0656	0.000455	0.000911	1.41%	1.09%
80		4	0.0179	0.00777	0.028	0.0114	0.0266	0.00318	0.00635	35.5%	72.6%

**CETIS Summary Report**

Report Date:

24 Mar-17 11:15 (p 2 of 2)

Test Code:

128994 49760109 | 10-0748-1929

**OCSPP 850.4500 Algal Toxicity****Wildlife International****96h AUC Detail**

C- $\mu$ g ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	6.17E+3	6.79E+3	7.69E+3	8.58E+3	6.93E+3	7.67E+3	8.04E+3	8.56E+3
4		6.76E+3	8.01E+3	8.31E+3	8.30E+3				
8.3		8.33E+3	8.90E+3	9.20E+3	9.11E+3				
21		9.54E+3	1.02E+4	8.85E+3	9.18E+3				
40		6.03E+3	6.25E+3	6.36E+3	4.86E+3				
80		1.96E+2	9.72E+1	1.38E+2	1.06E+2				

**96h Cell Density Detail**

C- $\mu$ g ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	232	213	256	260	288	300	288	281
4		227	258	279	284				
8.3		300	314	310	321				
21		332	343	307	327				
40		252	244	270	220				
80		5.9	1.9	2.1	1				

**96h Growth Rate Detail**

C- $\mu$ g ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	0.064	0.0631	0.065	0.0652	0.0662	0.0666	0.0662	0.066
4		0.0637	0.0651	0.0659	0.0661				
8.3		0.0666	0.0671	0.067	0.0673				
21		0.0677	0.068	0.0669	0.0675				
40		0.0648	0.0645	0.0656	0.0634				
80		0.0266	0.0163	0.0172	0.0114				

## OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	07-4655-5436	Endpoint:	96h AUC	CETIS Version:	CETISv1.8.7
Analyzed:	24 Mar-17 11:12	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes
Batch ID:	01-6116-4317	Test Type:	Algal Cell Growth (96-h)	Analyst:	
Start Date:	11 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent:	Algal Culture Media
Ending Date:		Species:	Navicula pelliculosa	Brine:	
Duration:	NA	Source:	Canadian Phycological Culture Centre	Age:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	13.1%	21	40	28.98	

## Dunnett Multiple Comparison Test

Control	vs	C- $\mu$ g ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision( $\alpha$ :5%)
Negative Control	4		-0.718	2.43	987	10	0.9830	CDF	Non-Significant Effect
	8.3		-3.27	2.43	987	10	1.0000	CDF	Non-Significant Effect
	21		-4.63	2.43	987	10	1.0000	CDF	Non-Significant Effect
	40*		4.13	2.43	987	10	0.0010	CDF	Significant Effect
	80*		18.3	2.43	987	10	<0.0001	CDF	Significant Effect

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision( $\alpha$ :5%)
Between	235246800	47049360	5	107	<0.0001	Significant Effect
Error	9683252	440147.8	22			
Total	244930100		27			

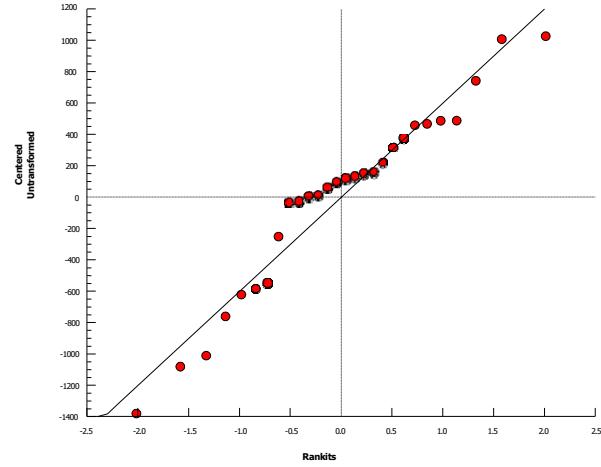
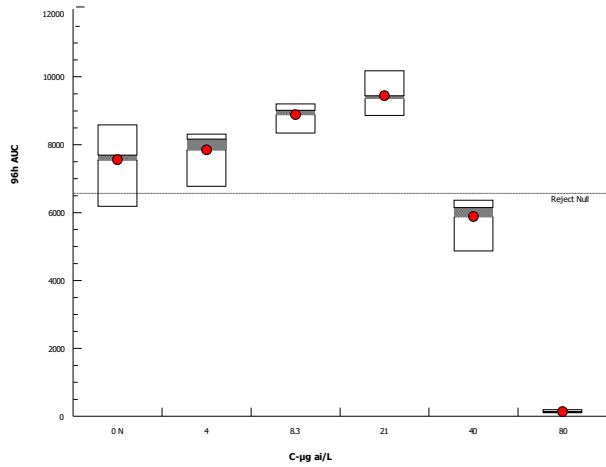
## Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision( $\alpha$ :1%)
Variances	Bartlett Equality of Variance	14.2	15.1	0.0147	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.951	0.897	0.2085	Normal Distribution

## 96h AUC Summary

C- $\mu$ g ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	7.55E+3	6.83E+3	8.28E+3	7680	6.17E+3	8.58E+3	3.05E+2	11.4%	0.0%
4		4	7.85E+3	6.68E+3	9.02E+3	8160	6.76E+3	8.31E+3	3.68E+2	9.38%	-3.86%
8.3		4	8.88E+3	8.27E+3	9.50E+3	9000	8.33E+3	9.20E+3	1.94E+2	4.38%	-17.6%
21		4	9.44E+3	8.54E+3	1.03E+4	9360	8.85E+3	1.02E+4	2.83E+2	6.0%	-24.9%
40		4	5.88E+3	4.78E+3	6.97E+3	6140	4.86E+3	6.36E+3	3.44E+2	11.7%	22.2%
80		4	1.34E+2	6.31E+1	2.05E+2	122	9.72E+1	1.96E+2	2.23E+1	33.3%	98.2%

## Graphics



## OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	02-3649-8417	Endpoint:	96h Cell Density	CETIS Version:	CETISv1.8.7
Analyzed:	24 Mar-17 11:12	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes
Batch ID:	01-6116-4317	Test Type:	Algal Cell Growth (96-h)	Analyst:	
Start Date:	11 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent:	Algal Culture Media
Ending Date:		Species:	Navicula pelliculosa	Brine:	
Duration:	NA	Source:	Canadian Phycological Culture Centre	Age:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	12.4%	40	80	56.57	

## Dunnett Multiple Comparison Test

Control	vs	C-µg ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control	4		0.207	2.43	32.8	10	0.8256	CDF	Non-Significant Effect
	8.3		-3.44	2.43	32.8	10	1.0000	CDF	Non-Significant Effect
	21		-4.62	2.43	32.8	10	1.0000	CDF	Non-Significant Effect
	40		1.36	2.43	32.8	10	0.3123	CDF	Non-Significant Effect
	80*		19.4	2.43	32.8	10	<0.0001	CDF	Significant Effect

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	282787.8	56557.55	5	116	<0.0001	Significant Effect
Error	10686.75	485.7616	22			
Total	293474.5		27			

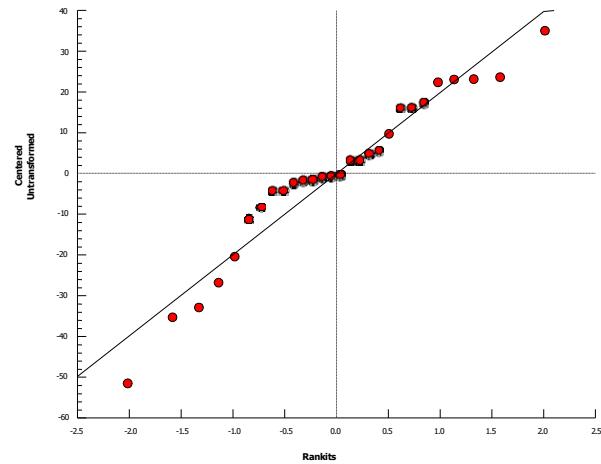
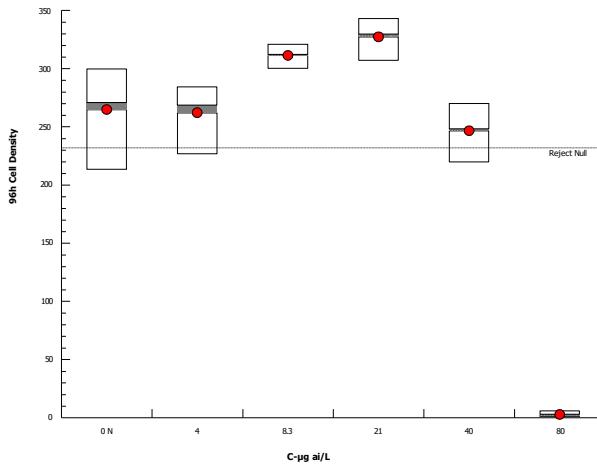
## Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	14.8	15.1	0.0113	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.949	0.897	0.1843	Normal Distribution

## 96h Cell Density Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	265	240	290	271	213	300	10.7	11.4%	0.0%
4		4	262	220	304	268	227	284	13.1	10.0%	1.06%
8.3		4	311	297	325	312	300	321	4.4	2.83%	-17.5%
21		4	327	303	351	329	307	343	7.61	4.65%	-23.6%
40		4	246	213	280	248	220	270	10.5	8.49%	6.91%
80		4	2.72	-0.728	6.18	2	1	5.9	1.09	79.6%	99.0%

## Graphics



## OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	13-0525-2850	Endpoint:	96h Growth Rate	CETIS Version:	CETISv1.8.7
Analyzed:	24 Mar-17 11:12	Analysis:	Nonparametric-Two Sample	Official Results:	Yes
Batch ID:	01-6116-4317	Test Type:	Algal Cell Growth (96-h)	Analyst:	
Start Date:	11 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent:	Algal Culture Media
Ending Date:		Species:	Navicula pelliculosa	Brine:	
Duration:	NA	Source:	Canadian Phycological Culture Centre	Age:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	4.26%	40	80	56.57	

## Mann-Whitney U Two-Sample Test

Control	vs	C- $\mu$ g ai/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision( $\alpha$ :5%)
Negative Control		4	19	NA	0	10	0.3232	Exact	Non-Significant Effect
		8.3	0.5	NA	1	10	0.9980	Exact	Non-Significant Effect
		21	0	NA	0	10	1.0000	Exact	Non-Significant Effect
		40	23	NA	0	10	0.1293	Exact	Non-Significant Effect
		80*	32	NA	0	10	0.0020	Exact	Significant Effect

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision( $\alpha$ :5%)
Between	0.007906073	0.001581215	5	251	<0.0001	Significant Effect
Error	0.0001383713	6.289602E-06	22			
Total	0.008044444		27			

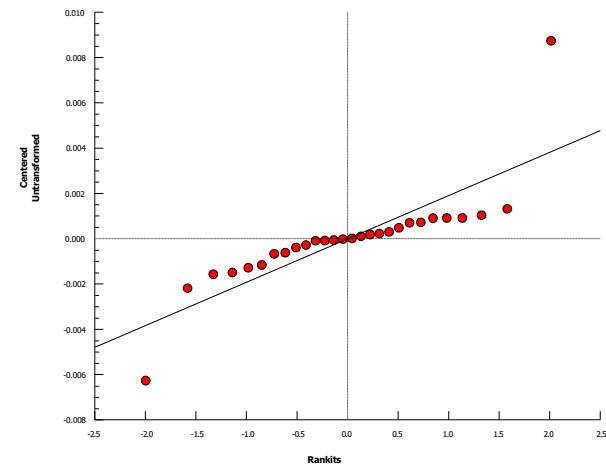
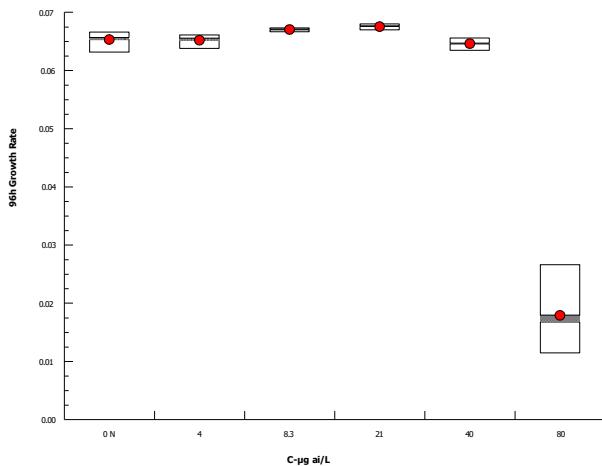
## Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision( $\alpha$ :1%)
Variances	Bartlett Equality of Variance	34.5	15.1	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.72	0.897	<0.0001	Non-normal Distribution

## 96h Growth Rate Summary

C- $\mu$ g ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	0.0653	0.0643	0.0663	0.0656	0.0631	0.0666	0.000432	1.87%	0.0%
4		4	0.0652	0.0635	0.0669	0.0655	0.0637	0.0661	0.000545	1.67%	0.13%
8.3		4	0.067	0.0665	0.0675	0.0671	0.0666	0.0673	0.000147	0.44%	-2.62%
21		4	0.0675	0.0668	0.0683	0.0676	0.0669	0.068	0.000232	0.69%	-3.43%
40		4	0.0646	0.0631	0.066	0.0646	0.0634	0.0656	0.000455	1.41%	1.09%
80		4	0.0179	0.00777	0.028	0.0168	0.0114	0.0266	0.00318	35.5%	72.6%

## Graphics



# CETIS Analytical Report

Report Date: 24 Mar-17 11:14 (p 1 of 6)  
 Test Code: 128994 49760109 | 10-0748-1929

## OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	03-2633-9845	Endpoint:	96h AUC	CETIS Version:	CETISv1.8.7
Analyzed:	24 Mar-17 11:12	Analysis:	Nonlinear Regression	Official Results:	Yes
Batch ID:	01-6116-4317	Test Type:	Algal Cell Growth (96-h)	Analyst:	
Start Date:	11 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent:	Algal Culture Media
Ending Date:		Species:	Navicula pelliculosa	Brine:	
Duration:	NA	Source:	Canadian Phycological Culture Centre	Age:	

### Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

### Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
12	-204	414	417	0.9052	Yes	8.95	3.05	0.0005	Significant Lack of Fit

### Point Estimates

Level	µg ai/L	95% LCL	95% UCL
IC5	31.2	N/A	39.5
IC20	37.5	28.7	42.4
IC50	45.5	32.1	64.5

### Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	8260	208	7850	8660	39.7	<0.0001	Significant Parameter
C	0.229	0.221	-0.205	0.663	1.04	0.3104	Non-Significant Parameter
D	45.5	5.83	34.1	56.9	7.81	<0.0001	Significant Parameter

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	2.23E+08	2.23E+08	1	260	<0.0001	Significant
Lack of Fit	11823440	3941146	3	8.95	0.0005	Significant
Pure Error	9683252	440147.8	22			
Residual	21506690	860267.5	25			

### Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	14.2	11.1	0.0147	Unequal Variances
	Mod Levene Equality of Variance	1.16	2.66	0.3620	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.959	0.926	0.3361	Normal Distribution
	Anderson-Darling A2 Normality	0.675	2.49	0.0781	Normal Distribution

### 96h AUC Summary

C-µg ai/L	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	7.55E+3	6.17E+3	8.58E+3	3.05E+2	8.63E+2	11.4%	0.0%
4		4	7.85E+3	6.76E+3	8.31E+3	3.68E+2	7.36E+2	9.38%	-3.86%
8.3		4	8.88E+3	8.33E+3	9.20E+3	1.94E+2	3.89E+2	4.38%	-17.6%
21		4	9.44E+3	8.85E+3	1.02E+4	2.83E+2	5.67E+2	6.0%	-24.9%
40		4	5.88E+3	4.86E+3	6.36E+3	3.44E+2	6.89E+2	11.7%	22.2%
80		4	1.34E+2	9.72E+1	1.96E+2	2.23E+1	4.46E+1	33.3%	98.2%

## OCSPP 850.4500 Algal Toxicity

Wildlife International

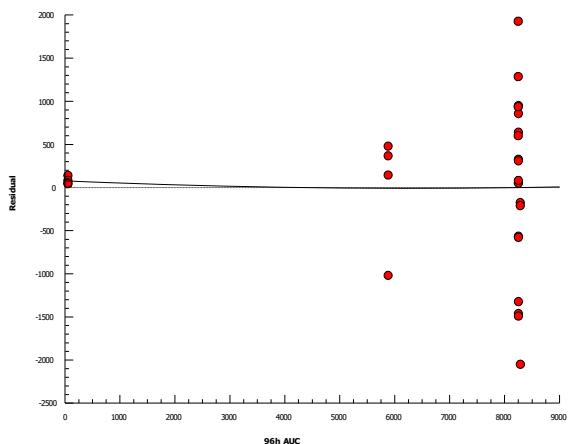
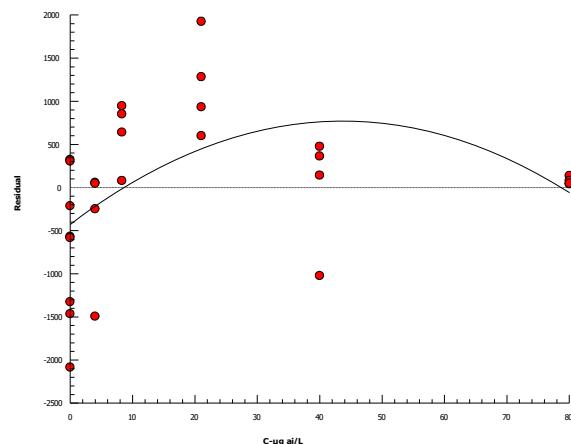
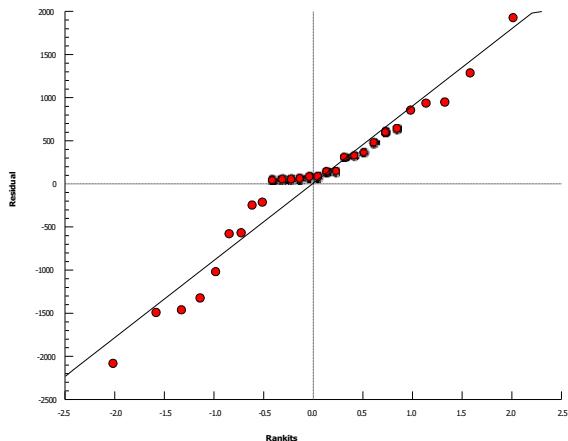
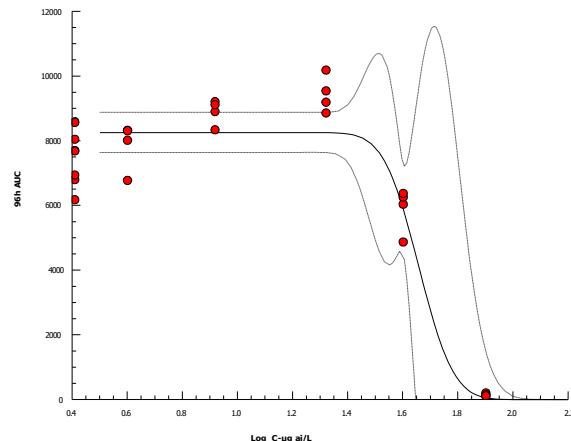
Analysis ID: 03-2633-9845  
 Analyzed: 24 Mar-17 11:12

Endpoint: 96h AUC  
 Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7  
 Official Results: Yes

## Graphics

3P Cumulative Log-Normal EV [Y=A\*(1- Φ(log(X/D)/C))]



**CETIS Analytical Report**

**Report Date:** 24 Mar-17 11:14 (p 3 of 6)  
**Test Code:** 128994 49760109 | 10-0748-1929

**OCSPP 850.4500 Algal Toxicity****Wildlife International**

<b>Analysis ID:</b> 01-0673-2768	<b>Endpoint:</b> 96h Cell Density	<b>CETIS Version:</b> CETISv1.8.7
<b>Analyzed:</b> 24 Mar-17 11:12	<b>Analysis:</b> Nonlinear Regression	<b>Official Results:</b> Yes
<b>Batch ID:</b> 01-6116-4317	<b>Test Type:</b> Algal Cell Growth (96-h)	<b>Analyst:</b>
<b>Start Date:</b> 11 May-15	<b>Protocol:</b> OCSPP 850.4500 Aquatic Plant (Algae)	<b>Diluent:</b> Algal Culture Media
<b>Ending Date:</b>	<b>Species:</b> Navicula pelliculosa	<b>Brine:</b>
<b>Duration:</b> NA	<b>Source:</b> Canadian Phycological Culture Centre	<b>Age:</b>

**Non-Linear Regression Options**

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

**Regression Summary**

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
5	-110	226	229	0.9046	Yes	10.5	3.05	0.0002	Significant Lack of Fit

**Point Estimates**

Level	µg ai/L	95% LCL	95% UCL
IC5	35.8	N/A	40.3
IC20	42	35.2	46.3
IC50	49.8	N/A	N/A

**Regression Parameters**

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	286	7.2	272	300	39.7	<0.0001	Significant Parameter
C	0.201	0.133	-0.0583	0.461	1.52	0.1411	Non-Significant Parameter
D	49.8	7.38	35.3	64.3	6.75	<0.0001	Significant Parameter

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	267552.4	267552.4	1	258	<0.0001	Significant
Lack of Fit	15235.35	5078.449	3	10.5	0.0002	Significant
Pure Error	10686.75	485.7616	22			
Residual	25922.1	1036.884	25			

**Residual Analysis**

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	14.8	11.1	0.0113	Unequal Variances
	Mod Levene Equality of Variance	2.45	2.66	0.0659	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.961	0.926	0.3776	Normal Distribution
	Anderson-Darling A2 Normality	0.536	2.49	0.1732	Normal Distribution

**96h Cell Density Summary**

C-µg ai/L	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	265	213	300	10.7	30.2	11.4%	0.0%
4		4	262	227	284	13.1	26.2	10.0%	1.06%
8.3		4	311	300	321	4.4	8.79	2.83%	-17.5%
21		4	327	307	343	7.61	15.2	4.65%	-23.6%
40		4	246	220	270	10.5	20.9	8.49%	6.91%
80		4	2.72	1	5.9	1.09	2.17	79.6%	99.0%

## OCSPP 850.4500 Algal Toxicity

Wildlife International

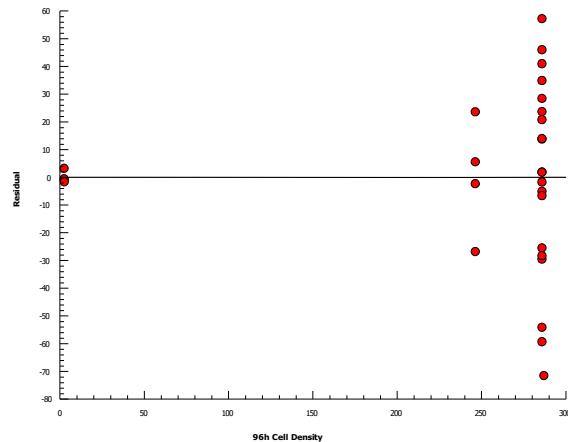
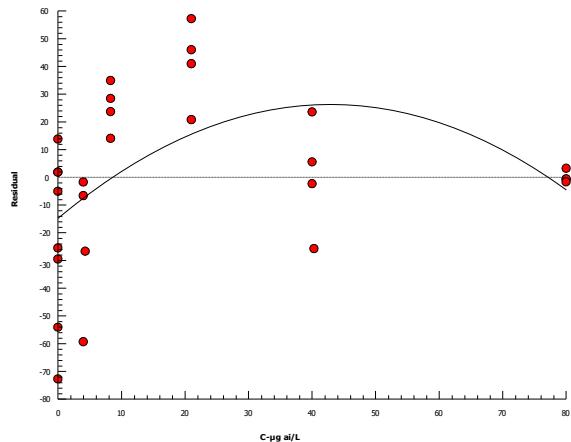
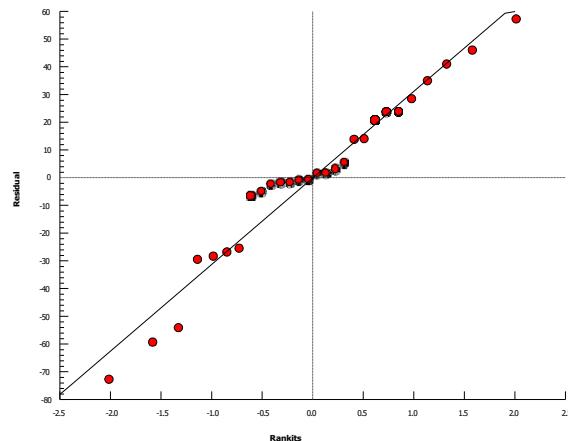
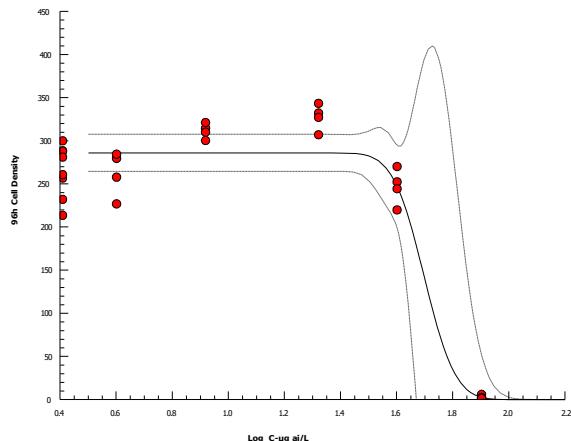
Analysis ID: 01-0673-2768  
 Analyzed: 24 Mar-17 11:12

Endpoint: 96h Cell Density  
 Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7  
 Official Results: Yes

## Graphics

3P Cumulative Log-Normal EV [Y=A\*(1- Φ(log(X/D)/C))]



# CETIS Analytical Report

Report Date: 24 Mar-17 11:14 (p 5 of 6)  
 Test Code: 128994 49760109 | 10-0748-1929

## OCSPP 850.4500 Algal Toxicity

Wildlife International

<b>Analysis ID:</b>	21-3852-7260	<b>Endpoint:</b>	96h Growth Rate	<b>CETIS Version:</b>	CETISv1.8.7
<b>Analyzed:</b>	24 Mar-17 11:12	<b>Analysis:</b>	Nonlinear Regression	<b>Official Results:</b>	Yes
<b>Batch ID:</b>	01-6116-4317	<b>Test Type:</b>	Algal Cell Growth (96-h)	<b>Analyst:</b>	
<b>Start Date:</b>	11 May-15	<b>Protocol:</b>	OCSPP 850.4500 Aquatic Plant (Algae)	<b>Diluent:</b>	Algal Culture Media
<b>Ending Date:</b>		<b>Species:</b>	Navicula pelliculosa	<b>Brine:</b>	
<b>Duration:</b>	NA	<b>Source:</b>	Canadian Phycological Culture Centre	<b>Age:</b>	

### Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

### Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
5	155	-303	-300	0.9788	Yes	1.05	3.05	0.3893	Non-Significant Lack of Fit

### Point Estimates

Level	µg ai/L	95% LCL	95% UCL
IC5	44	N/A	49.4
IC20	54.4	47.3	59.9
IC50	68	64.6	71.7

### Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	0.0661	0.000563	0.065	0.0672	117	<0.0001	Significant Parameter
C	0.265	0.0396	0.187	0.342	6.7	<0.0001	Significant Parameter
D	68	1.86	64.4	71.7	36.6	<0.0001	Significant Parameter

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	0.007886	0.007886	1	1250	<0.0001	Significant
Lack of Fit	1.99E-05	6.62E-06	3	1.05	0.3893	Non-Significant
Pure Error	0.000138	6.29E-06	22			
Residual	0.000158	6.33E-06	25			

### Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Mod Levene Equality of Variance	2.68	2.66	0.0488	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.828	0.926	0.0004	Non-normal Distribution
	Anderson-Darling A2 Normality	1.62	2.49	<0.0001	Non-normal Distribution

### 96h Growth Rate Summary

C-µg ai/L	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	0.0653	0.0631	0.0666	0.000432	0.00122	1.87%	0.0%
4		4	0.0652	0.0637	0.0661	0.000545	0.00109	1.67%	0.13%
8.3		4	0.067	0.0666	0.0673	0.000147	0.000294	0.44%	-2.62%
21		4	0.0675	0.0669	0.068	0.000232	0.000464	0.69%	-3.43%
40		4	0.0646	0.0634	0.0656	0.000455	0.000911	1.41%	1.09%
80		4	0.0179	0.0114	0.0266	0.00318	0.00635	35.5%	72.6%

**CETIS Analytical Report**

Report Date: 24 Mar-17 11:14 (p 6 of 6)  
Test Code: 128994 49760109 | 10-0748-1929

**OCSPP 850.4500 Algal Toxicity****Wildlife International**

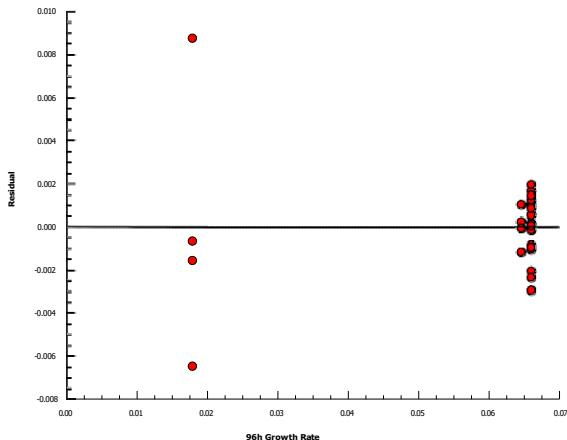
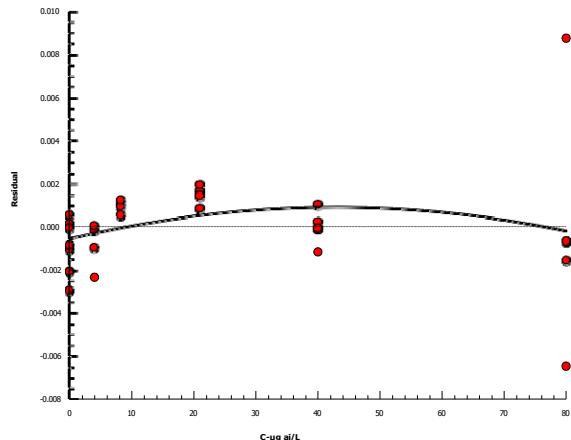
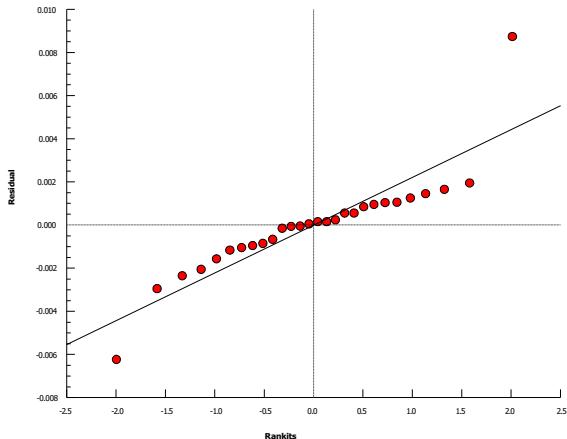
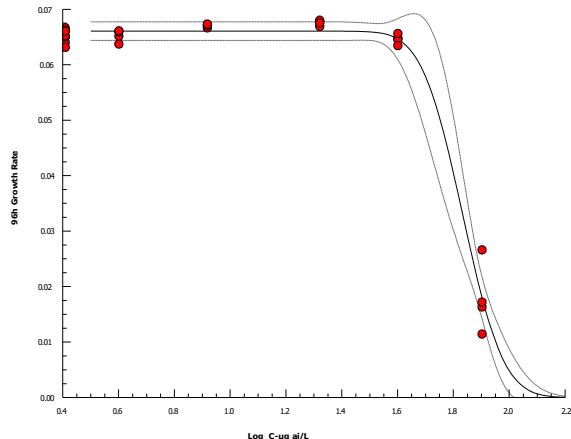
**Analysis ID:** 21-3852-7260  
**Analyzed:** 24 Mar-17 11:12

**Endpoint:** 96h Growth Rate  
**Analysis:** Nonlinear Regression

**CETIS Version:** CETISv1.8.7  
**Official Results:** Yes

**Graphics**

3P Cumulative Log-Normal EV [Y=A\*(1- Φ(log(X/D)/C))]



**CETIS Summary Report**

Report Date:

24 Mar-17 11:15 (p 1 of 2)

Test Code:

128994 49760109 | 10-0748-1929

**OCSPP 850.4500 Algal Toxicity****Wildlife International**

<b>Batch ID:</b> 01-6116-4317	<b>Test Type:</b> Algal Cell Growth (96-h)	<b>Analyst:</b>
<b>Start Date:</b> 11 May-15	<b>Protocol:</b> OCSPP 850.4500 Aquatic Plant (Algae)	<b>Diluent:</b> Algal Culture Media
<b>Ending Date:</b>	<b>Species:</b> Navicula pelliculosa	<b>Brine:</b>
<b>Duration:</b> NA	<b>Source:</b> Canadian Phycological Culture Centre	<b>Age:</b>
<b>Sample ID:</b> 20-8590-0954	<b>Code:</b> 128994 49760109	<b>Client:</b> CDM Smith - D. Worcester
<b>Sample Date:</b> 11 May-15	<b>Material:</b> Dithiopyr	<b>Project:</b>
<b>Receive Date:</b>	<b>Source:</b> Dow AgroSciences	
<b>Sample Age:</b> NA	<b>Station:</b>	

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
07-4655-5436	96h AUC	21	40	28.98	13.1%		Dunnett Multiple Comparison Test
02-3649-8417	96h Cell Density	40	80	56.57	12.4%		Dunnett Multiple Comparison Test
13-0525-2850	96h Growth Rate	40	80	56.57	4.26%		Mann-Whitney U Two-Sample Test

**Point Estimate Summary**

Analysis ID	Endpoint	Level	µg ai/L	95% LCL	95% UCL	TU	Method
03-2633-9845	96h AUC	IC5	31.2	N/A	39.5		Nonlinear Regression
		IC20	37.5	28.7	42.4		
		IC50	45.5	32.1	64.5		
01-0673-2768	96h Cell Density	IC5	35.8	N/A	40.3		Nonlinear Regression
		IC20	42	35.2	46.3		
		IC50	49.8	N/A	N/A		
21-3852-7260	96h Growth Rate	IC5	44	N/A	49.4		Nonlinear Regression
		IC20	54.4	47.3	59.9		
		IC50	68	64.6	71.7		

**96h AUC Summary**

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	7550	6830	8280	6170	8580	305	863	11.4%	0.0%
4		4	7850	6680	9020	6760	8310	368	736	9.38%	-3.86%
8.3		4	8880	8270	9500	8330	9200	194	389	4.38%	-17.6%
21		4	9440	8540	10300	8850	10200	283	567	6.0%	-24.9%
40		4	5880	4780	6970	4860	6360	344	689	11.7%	22.2%
80		4	134	63.1	205	97.2	196	22.3	44.6	33.3%	98.2%

**96h Cell Density Summary**

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	265	240	290	213	300	10.7	30.2	11.4%	0.0%
4		4	262	220	304	227	284	13.1	26.2	10.0%	1.06%
8.3		4	311	297	325	300	321	4.4	8.79	2.83%	-17.5%
21		4	327	303	351	307	343	7.61	15.2	4.65%	-23.6%
40		4	246	213	280	220	270	10.5	20.9	8.49%	6.91%
80		4	2.73	-0.728	6.18	1	5.9	1.09	2.17	79.6%	99.0%

**96h Growth Rate Summary**

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	0.0653	0.0643	0.0663	0.0631	0.0666	0.000432	0.00122	1.87%	0.0%
4		4	0.0652	0.0635	0.0669	0.0637	0.0661	0.000545	0.00109	1.67%	0.13%
8.3		4	0.067	0.0665	0.0675	0.0666	0.0673	0.000147	0.000294	0.44%	-2.62%
21		4	0.0675	0.0668	0.0683	0.0669	0.068	0.000232	0.000465	0.69%	-3.43%
40		4	0.0646	0.0631	0.066	0.0634	0.0656	0.000455	0.000911	1.41%	1.09%
80		4	0.0179	0.00777	0.028	0.0114	0.0266	0.00318	0.00635	35.5%	72.6%

**CETIS Summary Report**

Report Date:

24 Mar-17 11:15 (p 2 of 2)

Test Code:

128994 49760109 | 10-0748-1929

**OCSPP 850.4500 Algal Toxicity****Wildlife International****96h AUC Detail**

C- $\mu$ g ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	6.17E+3	6.79E+3	7.69E+3	8.58E+3	6.93E+3	7.67E+3	8.04E+3	8.56E+3
4		6.76E+3	8.01E+3	8.31E+3	8.30E+3				
8.3		8.33E+3	8.90E+3	9.20E+3	9.11E+3				
21		9.54E+3	1.02E+4	8.85E+3	9.18E+3				
40		6.03E+3	6.25E+3	6.36E+3	4.86E+3				
80		1.96E+2	9.72E+1	1.38E+2	1.06E+2				

**96h Cell Density Detail**

C- $\mu$ g ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	232	213	256	260	288	300	288	281
4		227	258	279	284				
8.3		300	314	310	321				
21		332	343	307	327				
40		252	244	270	220				
80		5.9	1.9	2.1	1				

**96h Growth Rate Detail**

C- $\mu$ g ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Negative Control	0.064	0.0631	0.065	0.0652	0.0662	0.0666	0.0662	0.066
4		0.0637	0.0651	0.0659	0.0661				
8.3		0.0666	0.0671	0.067	0.0673				
21		0.0677	0.068	0.0669	0.0675				
40		0.0648	0.0645	0.0656	0.0634				
80		0.0266	0.0163	0.0172	0.0114				